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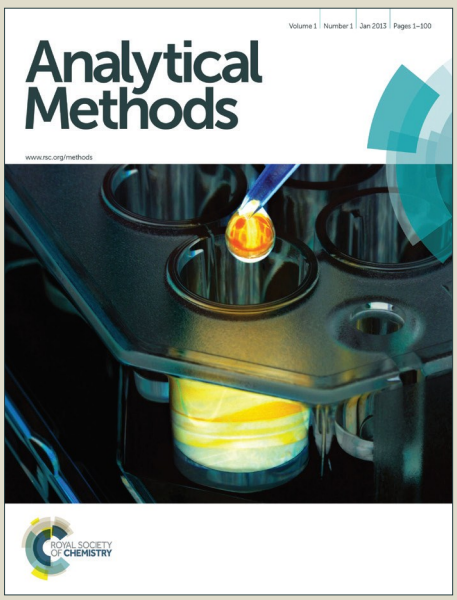

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Dispersive liquid-liquid microextraction coupled with single-drop microextraction for the fast determination of sulfonamides in environmental water samples by high performance liquid chromatography-ultraviolet detection

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ABSTRACT

A new model of fast and convenient liquid-liquid-liquid microextraction (LLLME), combining low-density solvent-based solvent-demulsification dispersive liquid-liquid microextraction (LDS-SD-DLLME) and single drop microextraction (SDME), was introduced to separate sulfonamides from environmental water samples for the first time. The extraction procedure includes a 2-min LDS-SD-DLLME fore extraction and a 15-min SDME back-extraction. A mixture of extraction solvent (1-octanol) and disperser solvent (methanol) was rapidly injected into the aqueous sample to form an emulsion for pre-extraction. Then a demulsifier solvent (acetonitrile) was injected into the extraction system. The emulsion turned clear in a few seconds and a layer of the organic phase formed at the top of the aqueous phase. At last a drop of acceptor solution was introduced into the upper layer and the SDME was carried out for the back-extraction. The whole procedure does not need any electric equipment (centrifuge, stirrer or ultrasonic cleaner) because the centrifugation in DLLME and the stirring step typically involved in SDME and LLLME are avoided by the successfully coupling of LDS-SD-DLLME and SDME. Four sulfonamides were firstly transferred from the donor phase to the organic phase by the LDS-SD-DLLME pre-extraction and then back-extracted into the acceptor droplet directly suspended in the upper layer of the organic phase. Factors affecting extraction efficiency were studied, including the organic solvent, the disperser solvent, the demulsifier solvent, the composition the of donor phase and acceptor phase, and the extraction time. At optimal conditions, the method showed low detection limit (0.22-1.92 μg/L) for the four sulfonamides, good linearity (from 1.0-500 to 10-500 μg/L, depending on the analytes) and repeatability (RSD below 4.6 %, n = 3). The simple, fast, and efficient feature of the proposed method was demonstrated by the analysis of sulfonamides in the lake

water, fishery water and wastewater samples.

Keywords: Liquid-liquid-liquid microextraction; Dispersive liquid-liquid microextraction; Single drop microextraction; Sulfonamide antibiotics; water samples.

INTRODUCTION

Sulfonamides (SAs) are commonly used in aquaculture and animal husbandry owing to their broad-spectrum activity and efficacy as growth promoters [1-2]. Ultimately, the residues of SAs can be excreted into the environmental soil and water [3-4]. Some of SAs can promote the development of antibiotic-resistant bacteria, cause allergic reactions in human, and even possess carcinogenic potential [5-6]. The content of SAs in untreated wastewaters ranges from 0.01 to 19.2 mg L^{-1}, and in treated wastewaters ranges from 0.004 to 6.0 mg L^{-1}, from a review of published data [7]. The European Union and US Food and Drug Administration (FDA) have provided that the total residues of SAs should not exceed 100 μg kg−1 in foodstuffs, such as fish, meat, eggs, milk and dairy products [8-9]. Therefore, there is a great need to monitor the trace of these compounds in environmental water.

In general, quantitative analysis of SAs are based on chromatographic techniques such as gas chromatography (GC) [10-12], capillary electrophoresis (CE) [13-15], and high performance liquid chromatography (HPLC) with ultraviolet (UV) [16-21], fluorescence detection (FLD) [22-27], and MS [28-31]. In the past 5 years, HPLC-MS(/MS) has become the most employed analytical technique for the determination of SAs due to its higher selectivity and sensitivity than other instrumental methods [7]. Nevertheless, HPLC-UV presents a cheap and effective method for the determination of SAs in many cases [16-21].

Prior to HPLC analysis, a relatively simple and effective preconcentration and clean-up pretreatment process is necessary to extract traces of SAs from the aqueous medium. Dispersive liquid-liquid microextraction (DLLME), as demonstrated advantages including rapidity, simplicity of operation, low cost, high recovery and enrichment factor [32], has been proposed to extract sulfonamides from water samples [8, 21, 25, 33].

Given that sulfonamide compounds are amphoteric and readily soluble in water, the liquid-liquid-liquid microextraction (LLLME) has been recommended for the preconcentration of

sulfonamides from water sample either using ionic liquid [34] or nitroxylene [1] as the organic phase. In this technique, pH adjustment in the donor phase can be used to control the hydrophilic-hydrophobic character of sulfonamides that provides good extractability for SAs. The organic phase may also play an efficient barrier to some interfering compounds coexisting in the aqueous phase. So the clean-up would be improved in this way. However, the conventional LLLME is usually a time-consuming technique. It was often observed that more time is needed to reach a good enrichment of the analytes of interest [1].

In response to this concern, we have developed a new format of LLLME by combining the low-density solvent-based DLLME with single-drop microextraction (SDME) for the fast and effective preconcentration of chlorophenols from environmental water samples [35]. The low-density solvent-based solvent-demulsification DLLME (LDS-SD-DLLME), being introduced in our previous work [36], has been well evaluated for the determination of carbamate pesticides [36] organochlorine pesticides [37] and polycyclic aromatic hydrocarbons (PAHs) [38, 39]. On the other hand, SDME is well known as a simple-operation liquid-phase microextraction (LPME) [40], although the instability of the suspending droplet often limits its application to various samples. The new DLLME-SDME combination [35] includes a 2-min DLLME pre-extraction and a 10-min SDME back-extraction. The acceptor droplet is directly introduced into the upper layer of low-density organic phase after the DLLME step. The high speed and efficiency of DLLME make the typical stirring step in SDME and LLLME unnecessary and the total extraction time noticeably short.

Here, low-density solvent-based solvent-demulsification DLLME combined with SDME was for the first time developed in a new format for the fast three-phase microextraction of trace sulfonamides in aqueous solution. In the proposed procedure, measured light organic solvent (1-octanol) and methanol (disperser) were rapidly injected into the aqueous sample (donor phase) and a cloudy solution was formed. After a 2-min pre-extraction, instead of mechanical centrifugation [35], a volume of demulsifier (acetonitrile) was employed to break down the emulsion. It cleared quickly to two layers in a few seconds after the injection of the demulsifier. Then a droplet of acceptor phase was introduced into the upper layer of the organic phase for the SDME back-extraction. The extreme simplicity, high speed and efficiency of the LDS-SD-DLLME-SDME coupling make the typical centrifugation in DLLME and stirring steps in SDME and LLLME unnecessary. Thereby simplifying the operation and

speeding up the pretreatment of samples. The developed method was applied to analyze several environmental water samples.

EXPERIMENTAL

Chemicals and supplies

Sulfathiazole (STZ), Sulfamethoxazole (SMX) and Sulfamethazine (SMZ) with purity of 99.0% were purchased from Sigma-Aldrich (Shanghai, China). Sulfanilamide (SN) with purity of 99.8% was supplied by Sinopharm (Shanghai, China). Structure, logD values and pKa values of target sulfonamides were shown in Table 1. Stock standard solutions of each analyte were prepared in methanol and stored at 4 ^{o}C. Mixtures of standard working solutions for extraction were prepared daily by diluting the stock standard solution with ultrapure water to the required concentrations.

Toluene, 1-octanol, decanol, n-hexane, cyclohexane, acetone, acetonitrile and methanol were purchased from Sinopharm (Shanghai, China). All reagents were of analytical grade or better. Ultrapure water was produced on a Milli-Q Academic water purification system (18.2 MΩ·cm, Millipore, USA).

Structure, logD values and pKa values of target sulfonamides, Sulfanilamide (SN), Sulfathiazole (STZ), Sulfamethazine (SMZ) and Sulfamethoxazole (SMX), were shown in Table 1.

The flat-cut needle tip of 10 μL microsyringe (Gaoge, Shanghai, China) was used for suspending the single drop of the acceptor phase. Disposable Teflon sleeve (0.7 mm i.d., 1.6 mm o.d.) was purchased from Agilent. The Teflon sleeve was cut into about 3 mm segments and replaced every new extraction. Before use, the sleeve was cleaned with acetone, methanol and water at least 10 times, respectively.

Instrumentation

Chromatographic analysis was performed with an Agilent 1200 HPLC system (Agilent, USA) including a ultraviolet-visible detector (VWD), a quaternary pump, a degasser and an analytical ChemStation. A Synergi Hydro-RP 80A C_{18} column (250 mm × 4.6 mm, 4 μm, Phenomenex, USA) was used for separation. The mobile phase used for separations was a binary solvent of acetonitrile : water (1% acetic acid). Gradient elution with a flow-rate of 1.0 mL min^{-1} was applied: initial 20% acetonitrile a linear ramp to 35% in 4 min, held at 35%. The detection wavelength was set at 265 nm and the analysis was carried out at 25 ^{o}C. The injection volume was 3 μL.

DLLME-SDME extraction procedure

The schematic procedure of LDS-SD-DLLME-SDME is shown in Figure 1. A volume of 7 mL aqueous sample (pH 4.5 adjusted by 0.05 mol/L NaH_2PO_4) containing analytes and 2 mol/L Na_2SO_4 was placed in a disposable polyethylene pipette (bottom: 55 mm height and 15 mm i.d.; top: 45 mm height and 7.5 mm i.d.) [35]. A mixture of 200 μL 1-octanol (as extraction solvent) and 750 μL methanol (as disperser solvent) was injected rapidly into the aqueous sample through a syringe. An emulsion of the extraction solvent, disperser solvent, and aqueous sample was formed in the pipette. After a 2-min pre-extraction, an aliquot of 600 μL acetonitrile serving as the demulsifier was injected into the pipette to break down the emulsion. The mixture cleared and turned to two layers within a few seconds.

The acceptor solution (0.1 mol/L NaOH) was taken by means of a 10 μL microsyringe fitted with Teflon sleeve. The microsyringe was lowered down vertically and slowly until the tip of the needle was barely immersed in the upper layer of the organic phase at the narrow stem of the pipette. The acceptor solution was pushed forward to the end of the microsyringe needle and a 3 μL droplet was suspended at its tip. After a 15-min back-extraction, the acceptor droplet was retracted into the microsyringe and manually introduced to HPLC system for further analysis.

Water samples and analytes

Water samples were collected from the South Lake and a fish pond, and a site of aquaculture drainage near the campus (HZAU, Wuhan, China). The samples were filtered through the 0.45 μm pore size membrane filters into glass bottles and stored in the dark at 4 ^{o}C until their analysis (within 72 h).

RESULTS AND DISCUSSION

Design of phase separation in DLLME

Typically, most DLLME procedures have a centrifugation step, which is somewhat time-consuming and needs a cooling setup in cases to ensure a good phase separation. Recently in our previous work, a solvent-termination (demulsification) step was validated to be an alternative design of phase separation in DLLME [36]. The performance of solvent-demulsification (1 mL acetonitrile as demulsifier) was compared with the centrifugation (at 3000 r min^{-1} for 2 min) for the separation of the dispersed organic phase and the aqueous phase. As demonstrated in Figure 2, peak areas for tested sulfonamides were higher when solvent-demulsification was used for phase separation than

centrifugation. Solvent-demulsification rather than centrifugation was selected for the phase separation in following experiments.

Extraction solvent and its volume

The selection of organic solvent was based on the following conditions: low water solubility, moderate solubility of target compounds in it, and having a lower density than water. Four low-density organic solvents with different polarity, namely toluene, iso-octanol, decanol and 1-octanol were examined for the extraction solvent. A series of experiments were performed to evaluate the extraction solvents using 500 μL methanol as dispersive solvent and 1000 μL acetonitrile as demulsifier solvent. In order to achieve equal final volume in the upper layer for different extraction solvents after DLLME, different initial volumes of organic solvents were served based on their solubility in the extraction system. As illustrated in Figure 3, the highest extraction efficiency was achieved with 1-octanol for most sulfonamides. Therefore 1-octanol was selected as the organic phase.

The volume of the extraction solvent is an important parameter in DLLME which may influence the microextraction and the enrichment of the analyte. The volume of about 10-50 μL for the extraction solvent was usually used in conventional DLLME, whereas here the volume of 1-octanol should be large enough to facilitate implementing the SDME back-extraction in the upper layer. Previous experiments [34] showed that the final volume of the organic layer should not be less than 200 μL in the extraction pipette. Otherwise the upper layer would be too thin to suspend an acceptor droplet in it. In this respect, the 1-octanol volume of 200 μL was adopted in the following experiments.

Donor pH and addition of salt

Since sulfonamides are ordinary ampholytes, the pH of the donor phase was adjusted to $pKa^{average}$ to make their neutral forms dominant in the aqueous phase [1]. As trial results indicated, the $pKa^{average}$ of sulfonamides, *i.e.* the average of pKa_1 and pKa_2 of the compounds, are in the range of 3.6 to 6.0 (Table 1). Accordingly, a 0.05 mol/L concentration of NaH_2PO_4 was contained in the sample solution to keep the pH value of the donor phase at 4.5.

The salting-out effect is often used to increase the partition coefficient of the polar analytes to the organic phase in liquid-liquid extraction. In the same time, salting-out phenomenon would also reduce the solubility of the organic solvent in the donor phase, accelerating the phase separation of the organic

phase and the bulk sample after extraction. To this purpose, 1 mol/L of Na_2SO_4 (or NaH_2PO_4) was added in the sample solution, respectively, to investigate the salting-out effect on the extraction efficiency. As can be seen from Figure 4-A, higher extraction efficiency was obtained when Na_2SO_4 was added.

The solubility of Na_2SO_4 in water varies with temperature and 2 mol/L Na_2SO_4 is almost saturated in the water samples at room temperature. Then the salt addition experiment was further investigated with 0.5-2 mol/L of Na_2SO_4 adding to the aqueous sample. The obtained results (Figure 4-B) showed that the extraction efficiency of sulfonamides steadily increased with the content of Na_2SO_4 increasing. So 2 mol/L Na_2SO_4 was added into the sample solution.

Disperser solvent and its volume

The disperser solvent should be miscible between an organic phase and donor phase. Acetonitrile, acetone and methanol were often suggested being applied as disperser in DLLME. Both acetonitrile and acetone worked well as disperser with a low content of salt in the sample solution. Nevertheless, when 2 mol L^{-1} Na_2SO_4 was added into the aqueous sample, neither acetonitrile or acetone could lead to a good emulsion of extraction solvent and the donor phase. Sometimes the clouding solution could not even be observed. This phenomenon was explained by a remarkable intensification of the ionic strength of the aqueous phase by adding high content of salt into it.

Similar observation to the previous experiment [36] was obtained that methanol performed much better than acetonitrile and acetone as the disperser solvent. A series of volumes of methanol ranging in 250-1250 μL were investigated. The experimental results showed that 750 μL methanol is a suitable choice to ensure a good dispersion.

Demulsifier solvent and its volume

In the low-density solvent-based solvent-demulsification DLLME procedure, the water-miscible organic solvent methanol, acetonitrile and acetone would also be used as chemical demulsifiers to break down the dispersed system [36]. So the three commonly used solvents were evaluated in this work. However, contrast to the above observation of them acting as disperser, both acetonitrile and acetone performed better than methanol in this section as showed in Figure 5-A. The reason may be attributed to their characteristics of low surface tension and high surface activity. Acetonitrile was chosen as the

demulsifier solvent in following experiments.

Furthermore, the effect of the volume of acetonitrile as demulsifier solvent on the extraction of analytes was studied. Figure 5-B shows that higher extraction efficiency was obtained by using a larger amount of acetonitrile. On the other hand, excessive dosage of acetonitrile will cause more instability for the acceptor droplet suspended in the organic layer. Therefore, 600 μL acetonitrile was injected into the pipette to break down the emulsion.

Acceptor pH and volume

In order to efficiently extract sulfonamides into the acceptor phase, the pH of acceptor phase should ionize the trapped analytes to prevent them from being back-extracted into the organic phase. The alkaline acceptor phase was investigated using NaOH solution in the range of 0.005-0.5 mol/L. It can be seen from Figure 6 that 0.1 mol/L NaOH of acceptor solution presented satisfactory results. Thus 0.1 mol/L NaOH was used as the acceptor phase.

Size of the acceptor droplet plays an important role in back-extraction of analyte from the organic phase. The size also influences the enrichment factor by changing the volume ratio of the donor to the acceptor phase. Volume of 0.1 mol/L NaOH acceptor was examined in the range of 1-5 μL in a test trial. As the obtained results shown, larger droplets provided higher signal intensity of the analytes. More target molecules will move into the acceptor phase through the surface of the large droplet in a certain time than the small one. However, it was found that the NaOH droplets larger than 4 μL are unstable in the organic layer of 1-octanol. Subsequently 3 μL of acceptor phase using 0.1 mol/L NaOH solution was preferred in this work.

Extraction time

In the proposed method, the extraction consists of DLLME pre-extraction and SDME back-extraction. DLLME pre-extraction time means the time interval from the beginning of the dispersion and its end just before injection of the demulsifier solvent. The effect of DLLME time was examined in the range of 1-20 min. As showed in Figure 7-A, DLLME time longer than 2 min has no significant enhancement on the extraction efficiency of sulfonamides, because the rate of extraction in DLLME is extremely fast. In the following experiments, DLLME time of 2 min was adopted.

The effect of SDME back-extraction time on the extraction efficiency was examined in the range

of 2-20 min. As observed in Figure 7-B, the peak area of sulfonamides reached equilibrium after 15 min. It indicates that the mass-transfer of SDME back-extraction in this LLLME is noticeably faster than the conventional SDME. There are two reasons for this. The first is that a high concentration gradient of analyte in the organic phase to the acceptor phase has been contributed by the fast and effective DLLME pre-extraction. The second is that the volume ratio of the aqueous acceptor droplet to the organic donor layer is much larger than that in conventional SDME (the organic extraction droplet to the bulk of the aqueous solution). Because the volume of the 1-octanol layer here is only about 200 μL rather than 4-10 mL usually applied in conventional SDME for the volume of aqueous sample. Consequently, the SDME time was set at 15 min.

So the new LLLME combined a 2-min DLLME pre-extraction with a 15-min SDME back-extraction. Other suitable extraction conditions for the LDS-SD-DLLME-SDME method were as follows: the sample solution contained 0.05 mol/L NaH_2PO_4 (pH 4.5) and 2 mol/L Na_2SO_4; the extraction emulsion was generated by injection of 200 μL 1-octanol as extraction solvent and 750 μL methanol as disperser solvent into the aqueous phase and then demulsified by addition of 600 μL acetonitrile after 2 min of DLLME pre-extraction; a 3 μL droplet of 0.1 mol/L NaOH was served for the acceptor phase.

Method validation

The analytical performance of the proposed method under optimum conditions was validated through linearity (linear range and correlation coefficient), sensitivity (limits of detection), precision (expressed as relative standard deviation) and extraction efficiency (enrichment factors). The results are summarized in Table 2. The linear dynamic range (LDR) was 1 - 500 μg/L for SMZ and SMX, 5 - 500 μg/L for STZ, and 10 - 500 μg/L for SN, respectively, with the coefficient of determination (R^2) better than 0.9993. The limit of detections (LODs) for all target sulfonamides were calculated by the signal-to-noise (S/N) ratio of three and varied between 0.22 and 1.92 μg/L. The reproducibility was studied from five replicated experiments for spiked solution (50 μg/L SN, 50 μg/L STZ, 5 μg/L SMZ, 5 μg/L SMX). The intra-day relative standard deviation (RSD, n=5) was lower than 4.2% and the inter-day relative standard deviation (RSD, n=3) was lower than 4.6%. The enrichment factors of 6, 19, 55, and 91 for SN, STZ, SMZ, and SMX, respectively, were evaluated by comparing the calibration

graphs before and after the extraction process. It as can be seen from the Table 1 that the sequence of enrichment factors were in consistence with the logD values of the tested sulfonamides.

Comparison of the proposed technique with other microextraction techniques was presented in Table 3. As can be seen, LODs, RSD, LDR and EF of the presented method were comparable with the other methods in our comparison. In addition, the extraction time of the proposed method had a significant advantage compared with conventional LLLME methods.

Environmental water sample analysis

The procedure was applied to the analysis of sulfonamides in the lake, fishery and wastewater samples, and no target analytes were found in these samples. Then, spiked sulfonamides in real water samples were determined to assess the matrix effect. As given in Table 4, the relative recoveries of the targets were in the range of 85.9 % - 105.8 %. It demonstrated that the method was suitable for the determination of trace sulfonamides in the environmental water samples. The typical chromatograms of the non-spiked and spiked fishery water sample obtained by this method were shown in Figure 8 (spiking 5 μg/L for SMZ and SMX; 50 μg/L for SN and STZ).

Conclusion

In general, low-density solvent-based solvent-demulsification dispersive liquid-liquid microextraction (LDS-SD-DLLME) combined with single-drop microextraction (SDME) was developed and for the first time applied for the determination of sulfonamides in environmental water samples. The convenient LDS-SD-DLLME-SDME coupling avoids the typical centrifugation in DLLME, stirring step in SDME and LLLME, therefore the pretreatment does not need any electric device (centrifuge, stirrer or ultrasonic cleaner) in the whole extraction procedure, which simplifies the operation and speeds up the pretreatment of samples. The extreme simplicity, wiring needlessness, high speed and efficiency of the proposed method offers the opportunity to perform the sample pretreatments in the field.

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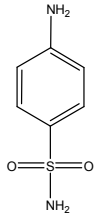
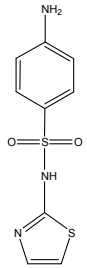
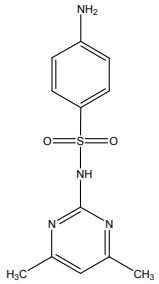
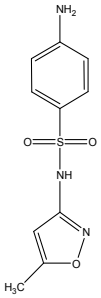
Tables

Table 1.

Structure, log D and pKa values of target sulfonamides

Sulfonamides	Sulfanilamide (SN)	Sulfathiazole (STZ)	Sulfamethazine (SMZ)	Sulfamethoxazole (SMX)
CAS No.	63-74-1	72-14-0	57-68-1	723-46-6
Structure				
logD	-0.67	0.045	0.29	0.64
pKa_1	1.85 ± 0.10	2.19 ± 0.10	1.69 ± 0.10	1.39 ± 0.10
pKa_2	10.10 ± 0.10	7.24 ± 0.10	7.89 ± 0.10	5.81 ± 0.50

LogD and pKa are calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (©1994-2011 ACD/Labs), which are from Scifinder Scholar.

Table 2.

Analytical characteristics of the proposed method for the determination of sulfonamides.

Analytes	Calibration curve (μg/L)	R^2	Linear range (μg/L)	LOD (μg/L)	EF	Intra-day RSD [a] %	Inter-day RSD [b] %
SN	y=0.0975x-0.5586	0.9993	10-500	1.92	6	4.2	4.0
ST	y=0.2096x-0.6529	0.9998	5-500	0.88	19	3.5	4.6
SM2	y=0.7258x-2.6558	0.9999	1-500	0.27	55	3.9	1.3
SMO	y=1.2073x-2.2077	0.9999	1-500	0.22	91	0.8	2.5

[a]: n=5, SN and STZ are 50 μg/L, SMZ and SMX are 5 μg/L.

[b]: n=3, SN and STZ are 50 μg/L, SMZ and SMX are 5 μg/L.

Table 3.

Comparison of the presented method for the determination of sulfonamides with other microextraction techniques.

Analyte	Method	Extraction time (min)	LOD (μg/L)	EF	Ref
STZ, SMZ, SMX	LLLME/AMADP[d]-HPLC-UV	45	0.11-0.77	-	1
SMZ, SMX	ILs-MADLLME[b]-HPLC-FLD	15	0.015-0.014	28-37	8
STZ, SMZ	Salting-out LLE-HPLC-UV	11	3.8-4.5	-	17
SMX	CPE-HPLC-UV	30	6.56	-	18
SMZ, SMX	HF-LPME-HPLC-UV	480	0.1-0.3	73-100	34
STZ, SMX	SPME-LC-MS/MS	50	14.0-26.3	-	41
SMZ, SMX	SBSE-LD[a]-HPLC/DAD	100	1.29-1.85	-	42
SMX	PPG_{400}-salt ATPS[c]-HPLC-FLD	40	0.1	-	43
SN, STZ SMZ, SMX	LDS-SD-DLLME-SDME[e]-HPLC-UV	17	0.88, 1.92 0.22, 0.27	6, 19 55, 91	This work

[a]: SBSE-LD: Stir bar sorptive extraction and liquid desorption.

[b]: ILs-MADLLME: Ionic liquids-based microwave-assisted DLLME.

[c]: PPG_{400}-salt ATPS: Poly (propylene glycol) $_{400}$-salt aqueous two- phase system.

[d]: LLLME/AMADP: LLLME in utilizing automated movement of acceptor and donor phase.

[e]: LDS-SD-DLLME-SDME: low-density solvent-based solvent-demulsification dispersive liquid-liquid microextraction single-drop microextraction.

Table 4.

Summary of recovery study performed on spiked water samples.

Analytes	Added (μg L^{-1})	South Lake water		Fishery water		Wastewater	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
SN	50	96.6	1.5	105.8	3.4	95.0	2.1
STZ	50	100.7	2.7	101.0	3.6	95.0	1.0
SMZ	5	95.2	4.6	99.3	4.8	85.9	6.2
	50	96.5	6.3	104.3	4.9	98.2	3.6
SMX	5	96.2	3.9	86.8	4.0	87.6	2.6
	50	99.7	2.1	102.1	6.1	94.2	3.1

Figure captions

Figure 1. The LDS-SD-DLLME-SDME procedure.

Steps (a) and (b) injecting extractant and disperser solvent into the donor phase (pH 4.5) that generates a cloudy solution; (c) adding acetonitrile to break down the emulsion; (d) after phase separation the organic phase going to the upper layer; and (e) suspending an acceptor droplet in the organic phase for back-extraction.

Figure 2. Effect of phase separation method on extraction of sulfonamides.

Aqueous sample: 500 μg/L SAs, 0.05 mol/L NaH_2PO_4, no Na_2SO_4; Organic solvents: 500 μL methanol and 200 μL 1-octanol; Acceptor solution: 3 μL 0.01 mol/L NaOH; Extraction time: 2 min DLLME, 10 min SDME.

Figure 3. Effect of extraction solvent on extraction of sulfonamides

Organic solvents: 250 μL toluene, 235 μL iso-octanol, 225μL decanol, or 235 μL 1-octanol; other conditions are same to Figure 1.

Figure 4. Effects of salt addition (A) and Na_2SO_4 concentration (B) on extraction of sulfonamides.

Figure 5. Effect of demulsifier solvent (A) and acetonitrile volume (B) on extraction of sulfonamides

Sample solution: 500 μg/L SAs, 0.05 mol/L NaH_2PO_4, 2 mol/L Na_2SO_4; Organic solvents: 750 μL methanol and 200 μL 1-octanol; Acceptor solution: 3 μL 0.1 mol/L NaOH; Extraction time: 2 min DLLME, 10 min SDME.

Figure 6. Effect of NaOH concentration on extraction of sulfonamides.

Sample solution: 500 μg/L SAs, pH 4.5, 0.05 mol/L NaH_2PO_4, 2 mol/L Na_2SO_4; Organic solvents: 750 μL methanol and 200 μL 1-octanol; Acceptor solution: 3 μL 0.1 mol/L NaOH; Extraction time: 2 min DLLME, 15 min SDME.

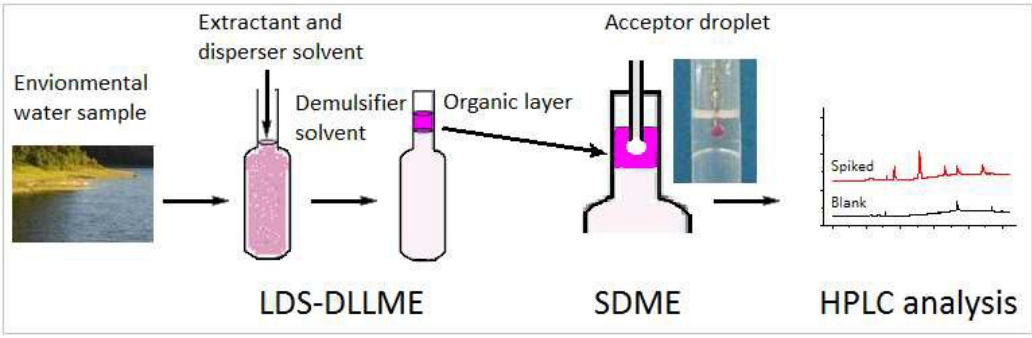
Figure 7. Effect of DLLME time (A) and SDME time (B) on extraction of sulfonamides.

Figure 8. Chromatograms the non-spiked (blank) and the spiked fishery water sample.

Sample solution: pH 4.5, 0.05 mol/L NaH_2PO_4, 2 mol/L Na_2SO_4; spiked sample: 5 μg/L for SMZ and SMX; 50 μg/L for SN and STZ. Organic solvents: 750 μL methanol and 200 μL 1-octanol. Acceptor solution: 3 μL 0.1 mol/L NaOH. Extraction time: 2 min DLLME, 15 min SDME.

Figures

TOC Art



A simple coupling of low-density solvent-based solvent-demulsification dispersive liquid-liquid microextraction (LDS-SD-DLLME, 2-min pre-extraction) and single-drop microextraction (SDME, 15-min back-extraction) was developed for the determination of sulfonamides in environmental water samples for the first time.

Figure 1

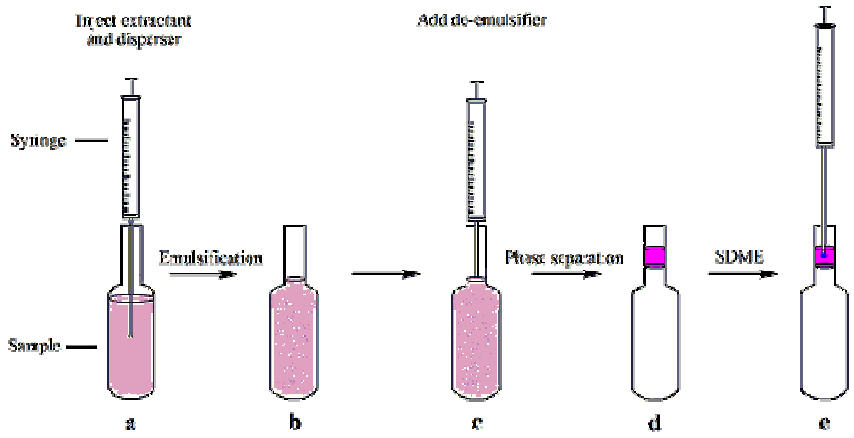
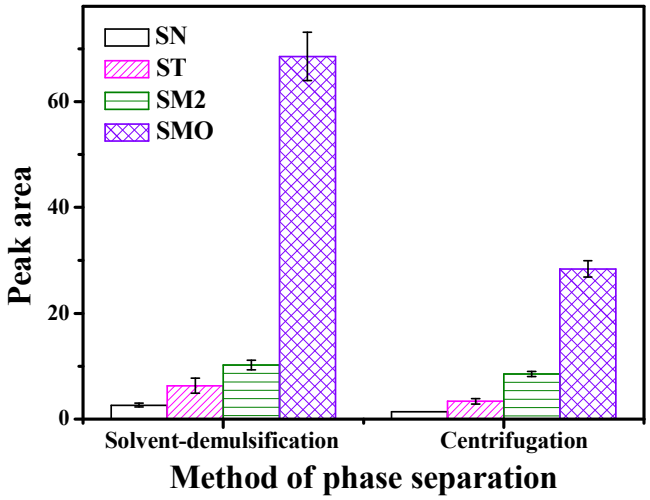


Figure 2



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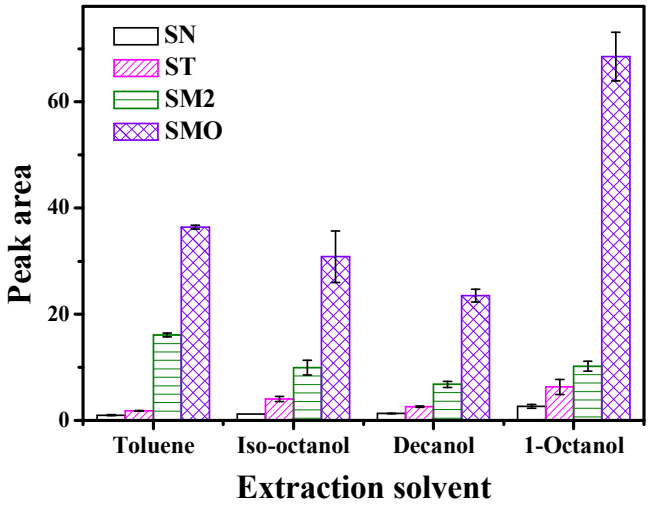
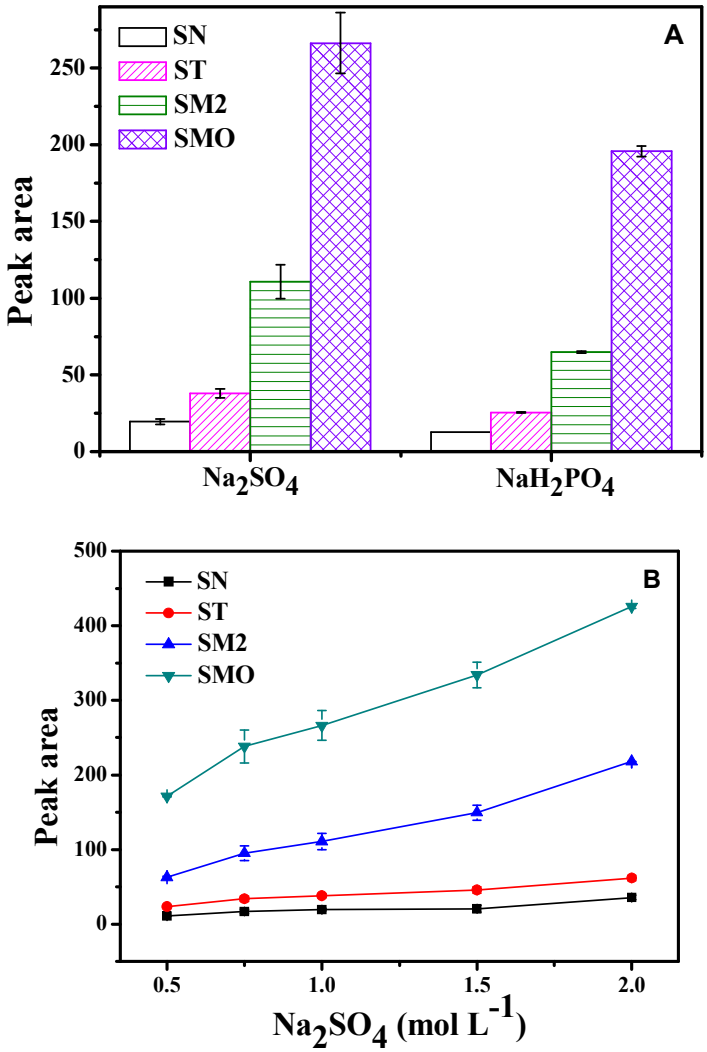


Figure 4



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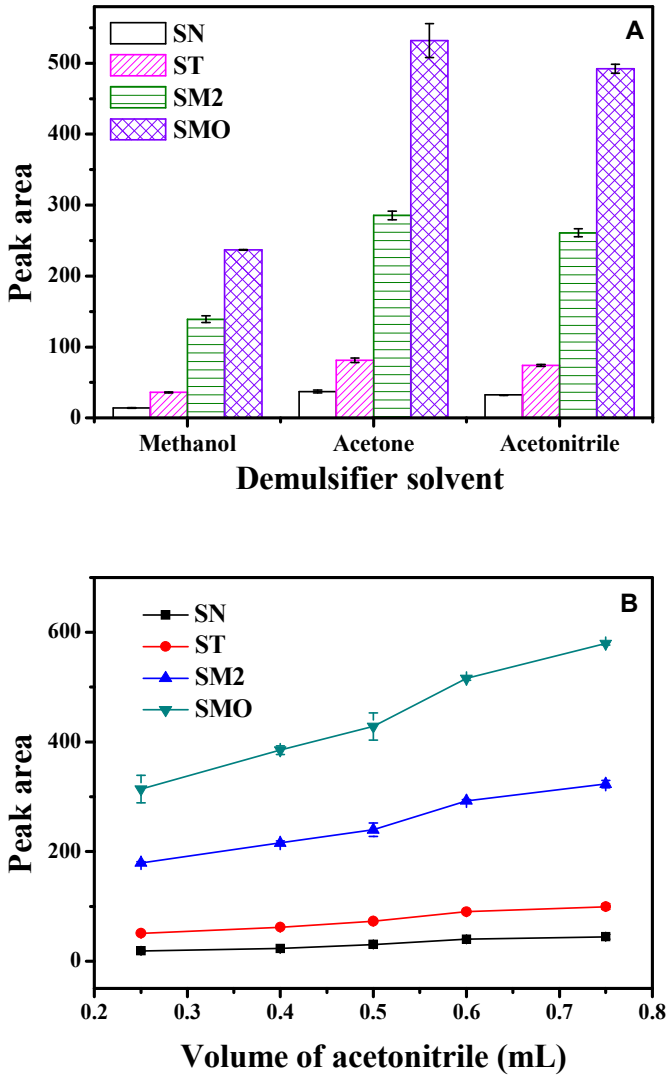
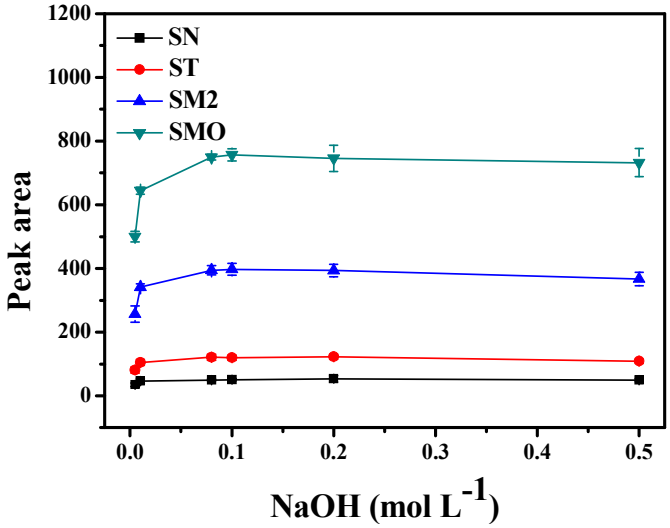


Figure 6



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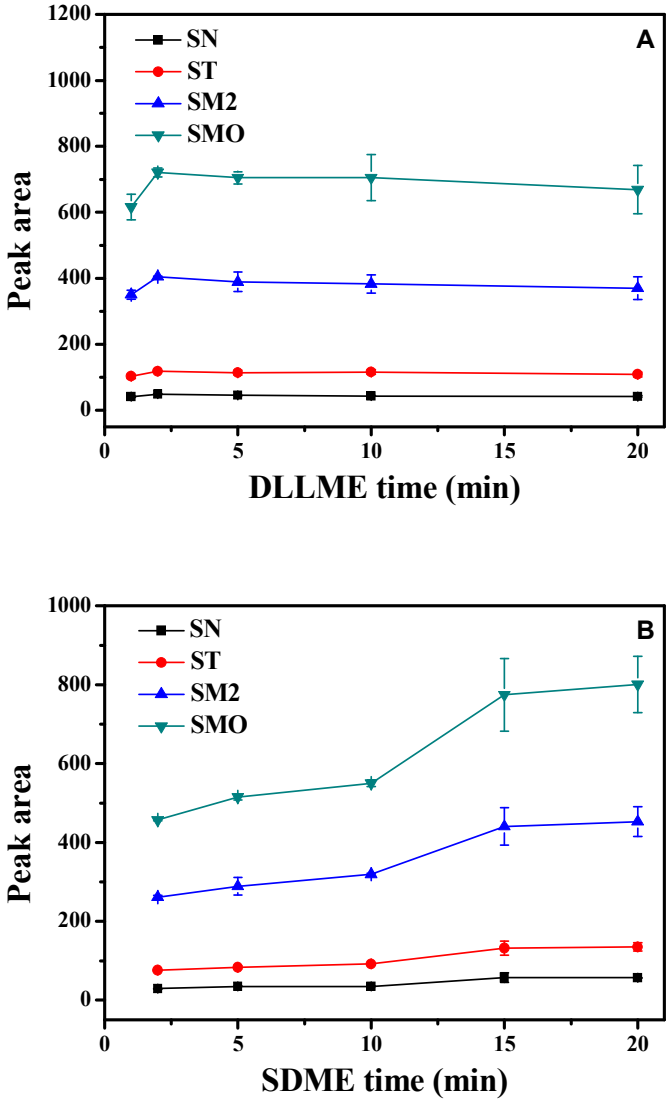

Figure 8